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Review

Molecular factors involved in the development of diabetic foot syndrome

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Diabetes is one of the major challenges of modern medicine, as it is considered a global epidemic of the XXI century. The disease often leads to the development of serious, health threatening complications. Diabetic foot syndrome is a characteristic set of anatomical and molecular changes. At the macroscopic level, major symptoms are neuropathy, ischemia and chronic ulceration of the lower limb. In every third patient, the neuropathy develops into Charcot neuroarthropathy characterized by bone and joints deformation. Interestingly, all these complications are a result of impaired healing processes and are characteristic for diabetes. The specificity of these symptoms comes from impaired molecular mechanisms observed in type 1 and type 2 diabetes. Decreased wound and fracture healing reflect gene expression, cellular response, cell functioning and general metabolism. Here we present a comprehensive literature update on the molecular factors contributing to diabetic foot syndrome.

Key words: Diabetic foot syndrome, Charcot neuroartropathy, molecular mechanisms, bone metabolism

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INTRODUCTION

Diabetes is one of the most deceitful diseases that affect more than 346 million people in the world (WHO organization 2012). Not only is the disease itself dangerous but it leads to many complications, like vascular damage and retinopathy (Pirola *et al.*, 2010). The incidence of these complications increases with age and duration of diabetes. Of all diabetic complications, diabetic foot is one of the most devastating and costly (Cornell & Dorsey, 2012). It develops as a result of neuropathy and ischemia. The risk of foot ulceration development as a consequence of above pathologies is as high as 25% (Singh *et al.*, 2005).

One of the outcomes of neuropathy is the development of insensitive foot, which increases the risk of ulceration. Degeneration of sensory innervation leads to insensitivity to pain caused by ulcers, wounds, or infections, which are the major causes of limb loss. Diabetic foot syndrome also includes Charcot arthropathy, which is a relatively rare complication of diabetes but studies report incidence of 0.15% up to nearly 2.5% (Gupta & Mohan, 2003; Vasquez & Henderson, 2010). It occurs in patients diagnosed with deep damage to the peripheral nervous system and intact circulation in the feet. It is believed that in the group of patients with neuropathy, about 16% may develop neuroarthropathy (Cavanagh *et al.*, 1994). Due to the lack of specific markers and a non-specific clinical picture of Charcot osteoarthropathy, as many as 25% of cases can be missed or the diagnosis may be delayed (Frykberg & Belczyk, 2008). Late diagnosis and lack of treatment may result in irreversible deformity of the foot, which is then prone to ulceration, infection and consequently may require amputation. Because of the limited treatment availability and serious consequences, diabetic foot syndrome is becoming an important issue. In the current review, we focused on the molecular basis of the diabetic foot syndrome. Our aim was to gather information about the factors that contribute to chronic ulceration and bone degeneration, and their function in the context of diabetes.

PATHOGENESIS OF NEUROPATHY

The mechanism behind the development of the diabetic neuropathy is complicated. However, angiopathy and hyperglycemia-induced metabolic changes play the major role. Long-term hyperglycemia causes metabolic disorders which further lead to activation of additional glucose metabolism pathways, in particular the polyol pathway (Yagihashi *et al.*, 2007). Severe changes

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Abbreviations: AGEs, advanced glycation end products; AGF, angiopoietin-related growth factor (angiogenesis factor); CGRP, calcitonin gene related peptide; CTGF, connective tissue growth factor; DCCT, Diabetes Control and Complications Trial; ECM, extracellular matrix; EGF, epidermal growth factor; FGF, fibroblast growth factor; HB-EGF, heparin-binding EGF-like growth factor; HSP, heat shock protein; HUVEC, human umbilical vein endothelial cells; ICAM-1, intercellular adhesion molecule 1; IGF, insulin-like growth factor; IGFBP, insulin-like growth factor (IGF)-binding protein; IL, interleukin; IRAK-1, interleukin-1 receptor-associated kinase; KGF, keratinocyte growth factor; M-CSF, macrophage colony-stimulating factor; miR-146a, microRNA 146a; miR-221, microRNA 221; MMP, matrix metalloproteinase; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NGF, nerve growth factor; NOS, nitric oxide synthase; NT-3, neurotrophin-3; OPG, osteoprotegerin; PDGF, platelet derived growth factor; RANK, receptor activator of nuclear factor κ B; RANKL, receptor activator of nuclear factor κ B ligand; SDF-1α, stromal-derived factor-1α; TGF-β, transforming growth factor β; TIMPs, tissue inhibitors of metalloproteinases; TNF-α, tumor necrosis factor alfa; TRAF6, TNF receptor-associated factor 6, E3 ubiquitin protein ligase; TRAIL, TNF-related apoptosis-inducing ligand; VEGF, vascular endothelial growth factor; WHO, world health organization.

in the polyol pathway lead to an increased accumulation of sorbitol in cells of nervous tissue. Sorbitol is an intermediate product of the glucose-fructose conversion. The reaction is catalyzed by the enzyme aldose reductase. Initially, glucose is reduced to sorbitol and sorbitol is then oxidized to fructose. In hyperglycemia, this process is intensified leading to accumulation of sorbitol in axons, followed by cellular water influx, swelling and Schwann cell damage (Yagihashi *et al*., 2007; Prashanth *et al*., 2010). Apart of the direct effect on nervous tissue, chronic hyperglycemia is correlated with vascular damage (Turner, 1998). Increased blood sugar levels may lead to epithelial cell dysfunction, which results in a decrease of pro-angiogenic signaling and production of nitric oxide (NO) (Lusis, 2000). NO additionally causes relaxation of smooth muscle, and its absence decreases vasodilatation, in consequence impairing blood supply to the nerve (Parashanth *et al*., 2010). Neuropathy in diabetes is correlated with cardiovascular damage not only resulting from metabolic defects, but caused by other risk factors such as hypertension, hyperlipidemia, obesity and cigarette smoking (Tesfaye *et al*., 2005). Moreover, micro and macroangiopathy have been identified as risk factors in type 2 diabetes mortality. Interestingly, microangiopathy is associated with poor glycemic control, and can develop as a consequence of hyperglycemia (Forsblom *et al*., 1998).

PHASES OF WOUND HEALING

The major reason for the limb amputation in diabetes is a high risk of sepsis. Infections develop due to chronic, non-healing wounds and ulcers (Bartus *et al*., 2004). To date, apart from the general diabetes care, the medical treatment of diabetic foot focuses mostly on proper wound treatment, with attention to regeneration enhancement, and fighting infections (Lipsky *et al*., 2012; Apelqvist, 2012). In the diabetic foot, the wound healing process is impaired or inhibited, which results in anatomical changes (Lobmann *et al*., 2005). Non-healing wounds may accelerate morbidity and mortality (Xu *et al*., 2012). The wound healing process depends on the general health of the patient, the location of the wound, age and accompanying diseases such as diabetes (Lobmann *et al*., 2005). The proper wound repair progresses through a number of ordered events. It is initiated by restoration of 1) hemostasis, followed by clot formation, 2) inflammation, 3) migration and cell proliferation determined by angiogenesis, granulation, tissue formation, epithelization and wound contraction, 4) cutaneous tissue remodeling (Blaktyny *et al*., 2006; Gethin, 2007; Guo & DiPietro, 2010).

NORMAL WOUND HEALING PROCESS

In general, wound healing depends on an interplay between different cell types and their contact mediated by soluble chemicals and hormones. In the wound site, platelets accumulate and aggregate, subsequently producing different growth factors responsible for the formation of the clot and matrix. Production of thrombin which initiates transformation of fibrinogen to fibrin, leads to the formation of a mesh stabilizing platelets at the site of injury. In addition, cytokines and growth factors permeabilize blood vessels, allowing for migration of leukocytes (Blaktyny *et al*., 2006).The leukocytes are attracted to the injured area by products of the decomposition of collagen and elastin, as well as cytokines TGF-β, TNF-α, interleukin-1 (IL-1) and platelet factor IV (Monaco *et al*., 2003). Accompanying neutrophils and macrophages remove contaminating bacteria and other foreign factors present at the wound site. Both neutrophils and macrophages release growth factors which initiate the formation of the tissue structure (platelet derived growth factor-PDGF and vascular endothelial growth factor-VEGF). Macrophages are responsible for releasing angiogenesis factor (AGF), which stimulates formation of new blood vessels. The processes of cell migration and proliferation are modulated by various factors, including epidermal growth factor (EGF) and keratinocyte growth factor (KGF-also known as FGF). Cell migration may also require the secretion of matrix metalloproteinases (MMP). This is necessary for partial degradation of the clot and the extracellular matrix (ECM) at the wound site. Partial digestion of the extracellular matrix forms space for the migrating cells. This process is tightly regulated and a balance between degradation and formation of new tissue has to be maintained (Madlener *et al*., 1998). On the other hand, collagen synthesis rebuilds the tissue and is stimulated by TGF (transforming growth factor), PDGF (platelet derived growth factor), and EGF (epidermal growth factor). Wound contraction, which is the last stage of the healing process, is an effect of the activity of mesenchymal cells (Blaktyny *et al*., 2006).

WOUND HEALING IN DIABETES

In diabetes, it has been observed that all stages of wound healing are affected in diabetic foot syndrome. This leads to a specific picture of diabetic wound, which is a hallmark of diabetic foot syndrome. It has been shown that a large number of growth factors, cytokines and chemokines, which are released by keratinocytes, fibroblasts, endothelial cells, macrophages and platelets, have their serum levels changed in diabetes. These factors are responsible for initiating the wound repair process and its maintenance, which results in gradual decrease in inflammatory response (Eubank & Marsh, 2010; Blaktyny *et al*., 2006). Diabetic wounds are characterized by a reduced level of the following growth factors and receptors: KGF (keratinocyte growth factor), TGF-β1 (transforming growth factor beta), NGF (nerve growth factor), PDGF, VEGF, IL-8, IL-10, IL-15, neurotrophin-3 (NT-3), substance P, CGRP (Blaktyny *et al*., 2009). Selected factors are described in Table 1. Decreased levels of these important growth factors might contribute to poor tissue regeneration and impaired wound healing.

EFFECTS OF TGF-β1 DEFICIENCY

Deficiency of TGF-β1 in chronic wounds may contribute to the increased level and activity of nitric oxide synthase (NOS). The enzyme produces nitric oxide (NO) which is a signal molecule with biological activity. The effects of NO signaling vary in different cell types and tissues. In general, it causes relaxation of smooth muscle, what leads to increased blood flow in blood vessels. However, during wound healing the contraction of blood vessel helps to prevent bleeding, thus NO action early during healing is unfavorable. Additionally its action is associated with prevention of platelet aggregation and leukocyte adhesion (Riddel & Owen, 1999). It is possible that increased levels of NO in the

plasma are associated with recurrent ulcers in diabetic foot syndrome (Blaktyny *et al*., 2006; Stojadinovic *et al*., 2012). The synthesis of NO positively correlates with expression of heat shock proteins (Hsps), while inhibition of the process reduces levels of Hsp (Mayshylev *et al*., 1995).

Abnormalities in the functioning of the heat shock proteins, which are also observed in diabetes, may lead to impairment of wound healing. In contrast to the increased Hsp levels observed in diabetic patients, rats with chemically induced diabetes show decreased levels of Hsp72 protein in blood. Interestingly, regulation occurs at the protein level since mRNA level is not affected (Bitar *et al*., 1999; Yamagishi *et al*.; 2001, Atalay *et al*., 2009). Current model explains the role of Hsp72 by its involvement in protein folding as well as in the transport of newly synthesized proteins required for tissue reconstitution (Kruglikov *et al*., 2011).

WOUND REPAIR THROUGH PROTEOLYSIS

Cell-to-cell communication is mediated by growth factors and cytokines secreted to the extracellular matrix (ECM). The dynamics of this extracellular environment is modulated by matrix metalloproteinases (MMPs) (McCawley & Martisan, 2001). These proteolytic enzymes are zinc-dependent endopeptidases (Maskos, 2004) Substrates for MMPs include structural elements of ECM (collagen, fibronectin, basal membrane), cell membrane receptors (CD44, ICAM-1, IGFBP) and growth factors precursors (pro-TNFα and precursor of TGFβ) (Nagase *et al*., 2006). Interestingly, mature forms of growth factors (CTGF and HB-EGF) and cytokines (RANKL and IL-1β) can also be substrates (Hao *et al*., 2003; Hashimoto *et al*., 2002; Zhang *et al*., 2003). Importantly, proteolysis may lead to activation or solubilization by release from ECM, as well as to degradation of the substrate (Visse & Nagase 2003, Nagase *et al*., 2006). It has been demonstrated that most MMP-encoding genes have a TGF-β1-dependent inhibitory element in the promoter region, which downregulates the gene's expression. Decreased levels of TGF-β1 lead to overexpression of MMPs, which results in excessive digestion of the growth factors. However, the molecular details of MMPs overexpression in diabetes are still not known (Stojadinovic *et al*., 2012). MMPs activity is modulated by tissue inhibitors of metalloproteinases (TIMPs), represented by four isoforms (Brew *et al*., 2000). These inhibitors enable tight regulation of protease activity preventing tissue destruction. This physiological balance between MMPs and their specific TIMPs is disrupted in diabetes (Lobmann *et al*., 2002; Stojadinovic *et al*., 2012). Several studies have shown a high concentration of MMP9 in wound liquid and a high ratio of MMP9 to TIMP-1 portends poor healing of diabetic foot ulcers (Ladwig *et al*., 2002; Liu *et al*., 2009).

The aspartic endopeptidase cathepsin D plays an important role in cell growth, proteolytic degradation, cell invasion and apoptosis (Beaujouin *et al*., 2006). Study in rats with streptozotocin-induced diabetes showed increased proteolytic degradation of collagen in wounds (Palka *et al*., 1991). This effect was reversed by external application of a cathepsin D inhibitor, suggesting that this was the main enzyme responsible for collagen decomposition. Interestingly, patients with ulcerated diabetic foot had increased plasma levels of cathepsin D, which can contribute to poor wound healing (Ahmad *et al*., 2012).

CYTOKINES INVOLVED IN WOUND HEALING

Cytokines are small proteins, soluble in the serum and interacting with cell surface receptors. They serve as a communication network between cells. By interaction with specific transmembrane receptor, they activate signal cascade, which result in cellular response (Nicholson *et al*., 2004).

Insuline-like growth factor (IGF) is a complex of two peptides: IGF-1 and IGF-2 (Jonathan *et al*., 2004). IGF-1 is a cytokine that participates in cell granulation during wound healing. During this process, expression of IGF-1 is increased. Interestingly, in diabetic patients expression of IGF-1 is decreased which may explain cell granulation defects (Yu *et al*., 2007; Blaktyny *et al*., 2005). It was found that IGF-1 is crucial in the regulation of the synthesis of Hif-1α protein during wound healing. The reduced level of IGF observed in diabetes may explain low levels of Hif-1a protein (Yu *et al*., 2007). Importantly, Hif-1α is an angiogenesis-promoting factor. It controls expression of several downstream factors involved in angiogenesis. Decreased levels of Hif-1α observed in diabetic mice and diabetic foot ulcers (Catrina *et al*., 2004; Botusan *et al*., 2008) can be one of the major reasons of poor blood vessel formation in diabetic foot. IGF-2 plays a key role in the development of the embryo and fetus. Shao and colleagues (2008) found that in fetuses of mice with induced diabetes, IGF-2 mRNA levels were reduced almost by half comparing with controls. It is possible that reduced level of IGF-2 is also associated with acute diabetes, since it is responsible for the development of pancreatic beta cells.

The process of wound healing in diabetic patients is also associated with a decreased level of expression of SDF-1α. Inhibition of SDF-1α activity leads to a reduction of the number of CD31+ cells which finally contributes to the much slower wound repair (Bermudez *et al*., 2011). CD31 is one of the markers analyzed in angiogenesis. It is a glycoprotein present on the surface of endothelial cells, monocytes, granulocytes and platelets (Scelsi *et al*., 2005). SDF-1α activity is the most important at early steps of wound healing, since its overexpression increases the healing rate (Badillo *et al*., 2007).

Analysis of skin biopsies at the molecular level reveals pathogenic markers that are associated with delayed wound healing. These include primarily an increased level of c-Myc. Its expression is activated by β-catenin, which is involved in the inhibition of keratinocyte migration, and EGF response (Stojadinovic *et al*., 2005).

Recent studies indicate a relationship between markers of inflammation IL-6, IL-8, IGF-1 levels and microvascular changes in the same group of patients with type I diabetes. Studies show that the level of IGF-1 is decreased in respect to control group and this is linked with increased levels of IL-6 and IL-8 in young people with type I diabetes (Abo El Asrar *et al*., 2012).

A very important factor that affects the inflammatory response is micro RNA miR-146a. This small non-coding RNA serves as a negative feedback regulator of *IRAK1* and *TRAF6* expression (Hou *et al*., 2009). IRAK1 and TRAF6 are elements of intracellular signal conducting cascade, and are associated with transmembrane receptors, including RANKL/RANK (Gohda *et al*., 2005). It has been shown that the miR-146a expression is significantly reduced in the wound in people diagnosed with diabetes. This correlates with an increased expression of its target genes *IRAK1* and *TRAF6*, as well as related elements of the signaling cascade of NF-κB, IL-6 and MP-2 (Xu *et al*., 2012). This situation leads to enhanced

Table 1. Molecular factors involved in diabetic foot syndrome formation

Factor	Status in diabetes	Effect	Reference
PDGF	decreased	decreased upon fracture healing, possible defects in general wound healing	Tyndal *et al*., 2003
KGF	decreased	healing rate decreased	Werner *et al*.,1994
VEGF	decreased	healing rate decreased	Frank *et al*., 1995, Bitto *et al*., 2008
IL-6 IL-8	increased	enhanced immune response, sustained inflammation state	Abo El Asrar *et al*., 2012
Igf-2	decreased	decrease observed in diabetic mice fetuses, possible implications in pancreatic cell development	Shao *et al*., 2008
Igf-1	decreased	cell granulation defects	Yu *et al*., 2007, Blaktyny *et al*., 2005
mir-146	decreased	enhanced immune response and sustained inflammation state	Xu *et al*., 2012
SDF1-α	decreased	slower healing rate, defects in angiogenesis	Badillo *et al*., 2007, Bermudez *et al*., 2011
c-Myc	increased	possible marker of B-catenin hyperactivation, resulting in inhibition of keratinocyte migration and EGF response	Stojadinovic *et al*., 2005
Hif1-α	decreased	possible defects in angiogenesis	Catrina *et al*., 2004, Botusan *et al*., 2008
RANKL	increased	disturbed RANKL/OPG ratio, possible increased bone resorption	de Amorin *et al*., 2008
TNFα	increased in wounds	enhanced fibroblasts apoptosis	Siqueira *et al*., 2010
TNFα TRAIL MCF	decreased in fractures	increased osteoclast activation and bone resorption and cartilage removal	Kayal *et al*., 2007; Kayal *et al*., 2009
cathepsin D	increased	abnormal collagen decomposition	Ahmad *et al*., 2012
MMP-9	increased	abnormal ECM decomposition, possible TGF1β degradation	Ladwig *et al*., 2002; Liu *et al*., 2009

inflammatory response, and can be a reason for chronic inflammation related to diabetes. Interestingly, treatment of wounds with mesenchymal stem cells reduces inflammation and accelerates wound healing. The treatment results in increased expression of miR-146a in the surrounding tissue, thus expression of miR-146a may depend on an extracellular signal (Xu *et al*., 2012).

FACTORS INVOLVED IN FRACTURE HEALING AND BONE REMODELING IN DIABETES

The disease process in diabetic foot syndrome can lead to severe destruction of bone structures. The cause of these changes is not clear. Diabetes may cause a net loss of bone due to the strong suppression of bone formation and accelerated bone resorption (Lu *et al*., 2003; Vestergaard *et al*., 2007). An increased bone-loss rate leads to development of osteopenia. It is characterized by the reduction of the mass of bone tissue, while retaining correct mineralization (Duarte *et al*., 2005).

It has been demonstrated that the molecular triad OPG/RANKL/RANK plays a major part in many bone-related diseases (Boyce *et al*., 2008). Osteoprotegerin (OPG) is a glycoprotein receptor from the family of tumor necrosis factor (TNF) produced in many organs of the body, including osteoblasts (Loris *et al*., 2001). It is present in a soluble form in the blood serum where its major role is capturing the excess of RANK ligand (RANKL). RANK protein is expressed in osteoclasts and their precursors. It is a membrane-anchored receptor present on the cell surface (Santini *et al*., 2011). RANKL is expressed mainly in bone and T cells, and plays an important role in the activation of osteoclasts (Santini *et al*., 2011; Boyce *et al*., 2008). RANKL binding to RANK receptor initiates a signal cascade mediated by TRAF6, leading to the activation and differentiation of the osteoclast which results in bone resorption. The effects of the interaction between RANKL and RANK are physiologically counterbalanced by OPG, which acts as a soluble receptor for RANKL and thus prevents the RANKL-RANK interaction. Any change in the ratio of RANKL to OPG may be critical in the control of the bone and skeletal system metabolism (Boyle *et al*., 2003).

It has been previously demonstrated in humans and in animal models that fracture healing in diabetes is much slower than in non-diabetic controls (de Amorin *et al*., 2008; Duarte *et al*., 2007; Diniz *et al*., 2008; Vestergaard *et al*., 2005). De Amorin and collegues (2008) studied bone remodeling in rats with alloxan-induced diabetes. Their results showed that 14 days after fracture expression of RANK, RANKL and OPG was reduced at fracture sites. However, the ratio of RANKL to OPG was clearly higher in the diabetic group, which may indicate increased bone resorption.

It was found that in rodents with type I diabetes the content of protein and minerals in the extracellular ma-

trix is reduced (Gandhi *et al*., 2006). It includes collagen, proteoglycan (PG), osteocalcin, calcium and magnesium. Additionally, the level of growth factors responsible for stimulating bone and cartilage formation IGF-1, PDGF, TGF-β1, basic fibroblast growth factor (bFGF), and VEGF is decreased. All these factors are essential for proper wound and bone healing, and their functional impairment is common in diabetes in humans (Bennett *et al*., 2003, Blaktyny *et al*., 2009).

Many factors that are specifically associated with osteoclast formation such as RANK, RANKL, tumor necrosis factor α (TNFα), TNF-related apoptosis-inducing ligand (TRAIL) and macrophage colony-stimulating growth factor (M-CSF) are upregulated in diabetic mice. This correlates with increased number of osteoclasts in tissues, resulting in increased bone and cartilage removal (Kayal *et al*., 2007; Kayal *et al*., 2009).

In the past few years, several studies have suggested that OPG and RANKL are linked with Charcot arthropathy (Jeffcoate, 2005; Mabilleau *et al*., 2008). It is a chronic disease characterized by progressive bone and joint destruction of the lower limbs. Symptoms are relatively painless due to a loss of the sensory innervation. The most characteristic symptoms include pathological fractures, joint dislocation, and deformations (Rogers *et al*., 2011). The majority of patients with Charcot neuroarthropathy are between 50 and 60 years old, and most of them have been suffering from diabetes for at least 10 years (Rajbhandari *et al*., 2002). A common feature of Charcot neuroarthropathy is increased bone reabsorption. It is most likely caused by increased activity of osteoclasts, which may lead to osteopenia (Pitocco *et al*., 2009). Additional evidence for a role of the molecular triad in Charcot neuroarthropathy development comes from genetic studies. It has been shown that selected polymorphisms in the *opg* gene occur more often in patients with diagnosed neuroarthropathy (Korzon-Burakowska *et al*., 2012).

Increased blood sugar levels in diabetics can lead to the formation of advanced glycation end products (AGEs) (Duarte *et al*., 2007), which are associated with delayed bone healing in these patients. Moreover, in diabetics this process may be escalated by the intensity of oxidative stress and the increased glucose levels (Santana *et al*., 2003). These conditions favor non-enzymatic glycation of proteins like hemoglobin, albumin, collagen and crystalline (Jabłońska-Trypuć, 2007). It is known that the accumulation of AGEs induces complicated changes in microdamage mechanism of bone (Tang *et al*., 2010) and leads to reduced mechanical strength of bone, which may cause fracture.

EPIGENETICS OF THE DIABETIC FOOT SYNDROME

Genetic mutations in factors directly involved in the diabetic foot syndrome are not solely responsible for the disease development. An increasing number of studies indicate the importance of epigenetic mechanisms in diabetes. Epigenetic changes are very diverse and include post-translational modifications of histones, methylation of cytosine in DNA, mitochondrial inheritance, microRNA expression, as well as transposable elements (Slotkin & Martienssen, 2007; Goldberg *et al*., 2007; Kim *et al*., 2009). These changes affect gene expression without changes in genomic DNA sequence. DNA methylation as the basis of genetic imprinting is a process of inactivation of one gene allele, depending on the methylation pattern inherited from one of the parents. In consequence, only one copy of the gene is active. As an example, the *IGF-2* gene is expressed only from the paternal allele (DeChiara *et al*., 1991). Large-scale studies of inherited methylation patterns and their association with disease identify several sites associated with type 2 diabetes (Kong *et al*., 2009). Among these, the most relevant is the *KCNQ1* locus, which has been associated with mono- and polygenic diabetes in humans (Yasuda *et al*., 2008; Unoki *et al*., 2008). Interestingly, DNA methylation patterns can also change in response to environmental stimuli. The process is termed metabolic memory, and is defined by changes of genes expression in cells exposed to various stress conditions (Pirola *et al*., 2010). It has been shown that methylation of adiponectin gene in the placenta results in elevated protein levels in mother's blood, and is inversely correlated with the mother's blood sugar levels (Bouchard *et al*., 2012). It is known that hyperglycemia is the most important factor contributing to diabetes-related complications, as revealed by large-scale, long-term studies DCCT and EDIC (Diabetes Control and Complication Trial 1993) (Nathan *et al*., 2005; Pop-Busui *et al*., 2009). In addition to the human studies, there is a number of animal studies (Engerman *et al*., 1987; Hammes *et al*., 1993; Kowluru *et al*., 2003) and *in vitro* cell culture studies (Roy *et al*., 1990; Ihnat *et al*., 2007) supporting the metabolic memory hypothesis. Especially a study performed by El Osta and colleagues (2008) shows changes in histone methylation in the *RELA* promoter region induced by hyperglycemia and maintained after restoring non-glycemic conditions. This kind of epigenetic mechanism might contribute to the overall picture of diabetic foot syndrome, changing expression of genes required for proper wound healing (Rafehi *et al*., 2012). However, despite numerous studies, there is no data about methylation patterns during wound healing process, or relation with diabetic foot syndrome. There are, however, several examples of micro RNA-mediated changes in gene expression (Madhyastha *et al*., 2011; Caporali *et al*., 2011). Some information can be obtained from *in vitro* studies on vascular epithelial cells (Li *et al*., 2009). Indeed, performed experiments show increased miR-221 RNA expression in HUVEC cells upon high glucose treatment. Treatment with miR-221 antisense RNA abolished the overexpression and restored the migration ability of the cells, which was previously impaired. However, the fact that epigenetic modifications can be induced metabolically argues for animal model studies. An interesting model for an epigenetic mechanism involved in neuropathy development comes from a study on HIV-associated-sensory neuropathy (HIV-SN) (Lehmann *et al*., 2011). HIV-SN is among the most frequent complications of HIV infection. It is characterized by a progressive anaesthesia starting in the feet (Cornblath *et al*., 1988). Interestingly, this neuropathy is caused by a deletion in the mitochondrial genome, leading to mitochondrial dysfunction in distal axons. It is possible that the neuropathy related to diabetic foot has a similar etiology.

SUMMARY

Diabetic foot syndrome is characterized by abnormalities at the macroscopic, cellular and molecular level. In this article, we reviewed the most important issues concerning cellular response and intracellular mechanisms affecting gene expression in the context of diabetic foot. The importance of the problem is highlighted by the increasing number of detailed studies. Attempts to understand the disease address different aspects of molecular biology, including the most recent findings about gene expression regulation. However, despite those extensive

studies in the field many questions remain unanswered. Future studies, although challenging, will bring more light into the field of diabetes-related complications, and most likely will result in effective therapies against diabetic foot syndrome.

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